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TLS in Tumors: What Lies Within

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Abstract

The role of Tertiary lymphoid structures (TLS) in cancer has recently been illustrated and revisited using mouse models. Joshi *et al.* describe regulatory T (Treg) cell infiltration and functional heterogeneity of TLS in lung tumors. Finkin *et al.* report that inflammation-associated TLS serve as niche for tumor progenitor cells, which may lead to recurrence in hepatocellular-carcinoma.

Tertiary lymphoid structures (TLS) are ectopic lymphoid formations that reflect lymphoid neogenesis occurring at sites of inflammation in non lymphoid tissues [1]. TLS exhibit characteristics of secondary lymphoid organs. They are composed of a T cell zone containing mature DC-Lamp+ dendritic cells (DC), adjacent to a B cell zone which includes a typical germinal center (GC) comprising B cells embedded within a network of follicular DC. High Endothelial Venules (HEV) surround TLS, enabling lymphocyte entry from the blood. TLS develop during infectious diseases, autoimmune and inflammatory disorders, graft rejection, in solid tumors and in inflamed tissues upon exposure to environmental stimuli. TLS density

correlates with protective outcome or disease exacerbation, depending on the context. In human cancers, TLS can be found in primary tumors [2] and in metastatic sites [1]. In all human cancer types tested so far, TLS density correlated with patient's favorable clinical outcome, and recent developments also suggest that TLS formation is associated with cancer patients' response to immunotherapy (reviewed in [1]). These observations, together with the coordinated CD8⁺ memory T cell and Th1 gene signature detected in the immune infiltrates of lung tumors with high TLS densities, have led to the concept that TLS allow the initiation and/or maintenance of a local and systemic adaptive immune response that protects patients against tumor invasion and metastasis [3]. However, animal models that could enable a comprehensive understanding of the function of TLS in cancer were lacking, with the exception of the signs of lymph node like-vasculature detected in B16 melanoma tumors [4]. Two recent articles using genetically engineered mouse models (GEMM) of lung cancer [5] and hepatocellular carcinoma (HCC) [6] shed light on TLS regulation and function and provide new insight on their role in cancer development.

Joshi *et al.* [5] investigated the relationships between Treg cells and TLS using a GEMM where lung adenocarcinoma is initiated by intratracheal instillation of lentiviruses expressing firefly luciferase fused to a portion of Ovalbumin and Cre recombinase that activates oncogenic *KRas* and deletes the tumor suppressor *Trp53* in lung epithelial cells. The authors observed that most tumors analyzed (93%) contained TLS similar to TLS detected in human cancers. In this model, tumor development is dependent on the presence of Treg cells: depletion of Treg cells leads to exacerbation of the disease and increased tumor infiltration by CD4⁺ and CD8⁺ T cells and macrophages. Joshi *et al.* found that most Treg cells in tumors were located within the Tumor-Associated (TA)-TLS, mainly in the T cell area. Ova-specific TCR transgenic CD8⁺T cells preferentially localized in the T cell areas of TA-TLS, formed immunological synapses with DC and proliferated. Localized depletion of Treg cells via intra-

tumor injection of diphtheria toxin (Treg cells carried a *Foxp3*-DTR allele) led to increased T cell proliferation in TA-TLS consistent with the idea that TLS-Treg cells regulate local anti-tumor responses. This evidence supports the notion that TA- TLS are sites where tumor-specific T cell responses are generated, and furthermore, that endogenous anti-tumor immune responses are inhibited by Treg cells within these structures. In accordance with these findings, elevated numbers of Treg cells in TLS correlate with poor survival of breast cancer patients [7].

Finkin *et al.* [6] developed an inflammation-driven HCC model by expressing constitutively the active form of IKK-B in hepatocytes to activate the NF-KB pathway, a situation that occurs in common forms of chronic hepatitis in humans [6, 8]. Similar to ectopic lymphoid structures (ELS) observed in human livers with hepatitis, ELS in these mice developed in the non-tumor zones of the inflamed liver, their aspect ranging from vague follicular aggregation to GC-containing follicles. Interestingly, small clusters of hepatocytes expressing E cadherin and several markers of HCC progenitor cells were found inside the ELS. These clusters progressively coalesced within the follicle boundary, egressed from the TLS and eventually grew as HCC in all IKK β expressing livers. Deletion of *Rag1* in IKK β □□□□□□□□□□□□□□□□ resulted in lack of ELS formation and attenuated hepatocarcinogenesis, suggesting that the formation of the ELS microniche for progenitor cells was dependent on the adaptive immune system. Lymphoid neogenesis is dependent on lymphotoxin receptor (LT β R) signaling. Analysis of the cells expressing LT β R in microdissected ELS revealed a switch from T and B lymphocytes in early forming structures to hepatocyte progenitors in advanced large ELS, suggesting that ELS provide paracrine LT β signals to early progenitor cells which could then be replaced by an autocrine signal. Injection of LT β R-Ig was associated with diminished intra-ELS progenitor cells and HCC burden. Analysis of ELS in human liver with hepatitis associated with human HCC revealed the

presence of HCC progenitor cells. In a series of 82 surgically treated patients with HCC [8], ELS density score, as well as intensity of expression of an ELS 12-gene signature comprising lymphoid and myeloid attracting chemokines, were associated with increased risk for late recurrence and a trend toward decreased overall survival after HCC resection (6).

These two studies suggest new roles for lymphoid structures in cancer. Joshi *et al.* demonstrate the presence of tumor-specific T cells within TA-TLS in an autochthonous mouse model of lung cancer, showing that TLS are sites for the generation of anti-tumor response immunity. A detailed characterization of Treg cell localization in cancers where the presence of Treg cells is associated with negative (Figure 1A) or positive [9] prognosis may provide insight into the roles of these cells during tumor progression. Finkin *et al.* show that hepatitis-associated ELS can provide a niche to support growth of tumor progenitor cells (Figure 1B). How to reconcile this result with the numerous lines of evidence that tumor-associated TLS correlate with good prognosis in human solid cancers? Of note, the same 12-gene signature associated with recurrence after surgical resection in HCC correlates with better patient survival in human colorectal carcinoma (10) suggesting that the tumor-associated TLS and the hepatitis-associated TLS share common features. Indeed one may postulate that ELS persisting in inflamed hepatic tissue at distance of the primary tumor have a different function than the anti-tumoral TLS developing during the slow process of tumorigenesis (Figure 1). In viral associated HCC TLS persist despite infection suggesting a loss of their protective role. Of note, Hepatitis C viral infection correlates with lymphomagenesis in some patients [11]. One major issue now is to find whether ELS niches for tumor progenitor cells can be found in other inflamed organs than the liver and be associated to cancer recurrence in other cancers than HCC, a concept which would need to re-visit the current literature. These findings add complexity to our understanding of TLS that prompts further study of these dynamic structures.

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Figure 1. Putative pro-tumoral roles for TLS in cancer. A. Treg located in Tumor Associated-TLS control the extent and activation CD4⁺ and CD8⁺ T cell infiltrates. TLS support a protective anti-tumor response (left). High TLS infiltration by Treg may correlate with tumor escape, in cancers where Treg are associated with poor prognosis (right). This situation

remains speculative since TA-TLS density is associated with good prognostic value in many human cancer types at different stages. B. ELS in non tumoral liver with hepatitis support growth of HCC progenitor cells, tumor recurrence and correlate with poor survival. Genes associated with inflammation including NFkB are expressed in the non tumoral liver (red). Hepatitis-associated ELS may serve as a “field” for carcinogenesis and provide a niche to produce de novo malignant tumors independently from the removed primary tumor (white).

